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(71) Applicants and
(72) Inventors: **YOUNG, Chung, Chang** [US/US]; 145 Buckskin Drive, Weston, MA 02493 (US). **CHAN, Joseph, L.** [US/US]; 139 Colonial Drive, Quincy, MA 02169 (US).

(74) Agents: **SHOPE, Suzanne, Seavello** et al.; Jones & Askew, LLP, 2400 Monarch Tower, 3424 Peachtree Road, N.E., Atlanta, GA 30326 (US).

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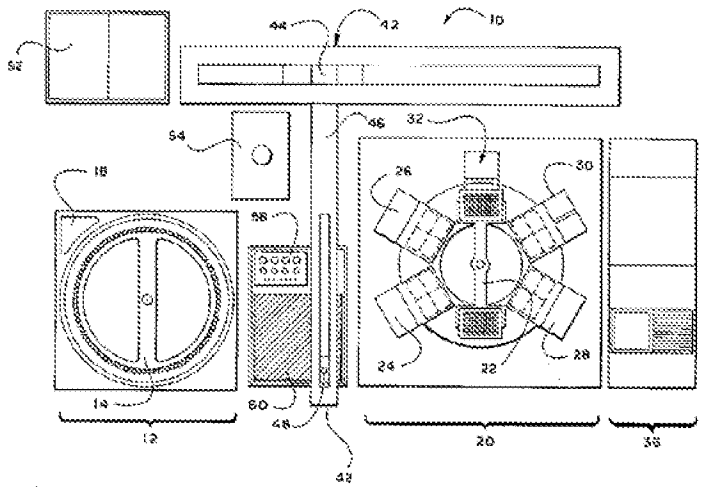

(57) Abstract: The analyzers of the present invention can be used for diagnostic applications of gene analysis by allowing flexible programming of thermal cycling, robotics, electrophoresis and reagents modules. The instrument is designed as three independent sub-systems, the nucleic acid sample preparation module, the nucleic acid manipulation module and the gel electrophoresis module. The system can be customized by removing any of these sub-systems, thus allowing for modification and future expansion. The system is fully automatic, including pre-analysis sample preparation (extracting nucleic acid from blood or tissue samples), manipulation of the nucleic acid (employing programmable incubators if needed), separation of the nucleic acid fractions using gel electrophoresis, analysis of the processed gels by an optical reader connected to the laboratory computer system having gel analysis software, and continuous sample identification from primary tube to result with a barcode reader.

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APPARATUS FOR FAST PREPARATION AND ANALYSIS OF NUCLEIC ACIDS

Field of the Invention

This invention relates to an apparatus for quick automatic analysis of biological samples. More particularly the present invention pertains to an apparatus for the automatic preparation, manipulation, and electrophoresis of nucleic acids from multiple biological samples for diagnostic analyses, such as, infectious disease diagnosis, identity testing, cancer diagnostics and predisposition testing.

Background of the Invention

There is no laboratory apparatus or equipment currently on the market that automates nucleic acid preparation, manipulation and detection in one, unattended operation. Devices for receiving biological specimens for diagnostic purposes are varied and adapted to the methods of detection. The devices may take the form of tubes for liquid specimens, flat surfaces such as glass slides suitable for microscopy, microtiter dishes, Petri dishes and cubes containing growth medium, or filters made of various materials to which cell and viral components will adhere.

These specimen samples are then treated in such a way as to indicate either the presence or absence, or quantity, of a specific biological entity. Test reagents may either be pre-applied to the device or added in series after the specimen is present. Test results are read manually by a technical person or automatically with instrumentation specifically designed for that assay. In some instances the specimen is diluted with a diluent, or an aliquot of the specimen is removed from the original collecting device and transferred to another vessel at some point in the assay. In some cases physical and chemical means are used to amplify the signal of the assay for greater sensitivity. Some assays require extraction or separation to isolate a specific component from other parts.

Pre-analysis sample preparation steps, such as, extracting the nucleic acid from a blood sample, can take one and one-half to two hours. This necessary but time-consuming step greatly adds to the cost to the consumer of each nucleic acid analysis.

In nucleic acid-based diagnostics the sequence specificity of base-pairing or enzymatic or other types of cleavage is exploited. The linear sequence of nucleotides in double-stranded DNA molecules forms the basis of replication of the genetic code. Hybridization is the binding of two single-stranded nucleic acid strands whose base-pairing sequences are complementary. Temperature and salt concentration affect the stringency of these base-pairing matches. A change from high stringency to low stringency can cause the same nucleic acid probe to be either specific to detect a particular target or less specific and detect a group of related targets.

Molecular biological techniques have provided many accurate, rapid tests for determining, identifying or detecting, DNA and RNA sequences. Amplification based technologies include the target amplification methods of nucleic acid sequence-based amplification (NASBA Organon Teknika), strand-displacement amplification (Becton Dickinson), transcription-based amplification system (TAS), transcription mediated amplification

(Genprobe), polymerase chain reaction (PCR, F. Hoffmann la Roche), and PCR *in situ*. Other amplification-based technologies include the signal (probe) amplification methods, such as ligase chain reaction (LCR/Abbott), q-beta bacteriophaqe replicase (Genetrak systems), cycling probe technology, (ID Biomedical of Vancouver), b-DNA (Chiron), *in situ* hybridization, (ligase hybridization, and genomic amplification with transcript sequencing (GAWTS). Reverse transcriptase-PCR (RT-PCR) indirectly analyzes RNA: the RNA is converted to a DNA copy (cDNA) and quantitative PCR is performed.

In some instances the sizes of DNA fragments, produced by restriction endonuclease digestion or by amplification of a target sequences between primer pairs, are used to make a DNA-print for individual identification or aid in diagnosis of a genetic disease, cancer or infectious disease. For example, electrophoresis may be used to size-fractionate different-sized nucleic acids which have been specifically cleaved or whose native length puts them in a distinguishable size-length class.

In the electrophoresis method, a current is applied to nucleic acid loaded at the cathodal end of a gel matrix, which causes the nucleic acid to migrate towards the anodal end of the matrix. The electrophoretic mobility of nucleic acid is dependent on fragment size and is fairly independent of base composition or sequence. Resolution of one size class from another is better than 0.5% of fragment size (Sealy P. G. and E. M. Southern. 1982. Gel electrophoresis of DNA, p. 39-76. In D. Rickwood and B. D. Hames (EDS.), Gel Electrophoresis of Nucleic Acids. IRL Press, London). This reference and all other publications or patents cited herein are hereby incorporated by reference.

Current nucleic acid analyzers, such as, _____ _____, are not automated. Each of the steps of sample preparation, manipulation, electrophoresis and data analysis is completed individually by technicians. Accordingly, these methods and apparatuses have a maximum

throughput of ten samples every three days. This slow data output is further undesirable because of high associated costs. Labor in processing the samples and analyzing the data from the biological samples requires four to five hours per day. Also undesirable, is the cost of the equipment to perform the analysis, which ranges from $250,000 to $350,000. All of these costs translate into high costs to consumers, within a range of $300.00 - $3,000.00 per nucleic acid analysis. Further compounding the problems of slow sample processing and high costs are significant problems with sample contamination, sample identity and labelling errors created when samples are transferred from one container to another, and variations in sample processing and data analysis between operators.

Accordingly, what is needed is an apparatus for fast, low cost, high volume, non-contaminated and consistent automated nucleic acid analysis of multiple biological samples.

What is also needed is an apparatus which is adaptable to dispensing different quantities of different reagents for saturating specimens quickly with a series of solutions automatically and wherein airflow and heating regulate and monitor temperature and humidity depending on the type of reactions necessary to complete the analysis desired.

Summary of the Present Invention

The process and apparatus of the present invention utilizes several state-of-the-art techniques to automate nucleic acid analysis directly from biological material. The apparatus employs modules for nucleic acid preparation, manipulation and hybridization, electrophoresis and data analysis, each interconnected by a computer system and robotic manipulators. The system of the present invention prepares nucleic acids from the samples for testing, sufficiently modifies nucleic acid sequences and accurately detects the presence or absence of diagnostic markers in the samples via hybridization and electrophoresis.

In a preferred embodiment shown in FIG. 1, the apparatus 10 of the present invention comprises three sub-systems. A first sub-system, the primary tube sampler 12, robotically prepares nucleic acid from biological samples. Connected to the first sub-system 12 is a second sub-system 20 for manipulating the nucleic acid sample. Connected to the first 12 and second 20 sub-systems is a third sub-system 36 for gel electrophoresis of the nucleic acid sample, whether the nucleic acid sample is modified or not by the second sub-system 20. The nucleic acid sample is mechanically transferred by the apparatus between the three sub-systems. The apparatus also comprises a means to mechanically pipette the biological samples, reagents used in the three sub-systems, waste generated in the three sub-systems, and the nucleic acids. The apparatus optionally also comprises a programmable central microprocessor for controlling the apparatus and analyzing data obtained by the apparatus. The three sub-systems are modular and each sub-system can be removed or added to these or other sub-systems.

The operation of the complete system begins with an operator placing one to 96 barcode labeled whole biological samples, such as blood, in a 96 position carrousel of primary tube sampler. Two empty 96 well titer plates are placed in the rotor of the plate centrifuge and indexer. One plate is used for the nucleic acid extraction while the other is used for the manipulation of the nucleic acid, such as PCR amplification. New gel cassettes are placed in the gel phoresis and reader system. Necessary reagent vials and a generous supply of pipette tips, for example, 2,000 tips, are placed in the storage system. The desired diagnostic test is chosen on the computer. The computer may provide instructions for checking or inserting reagents, such as enzyme solutions or probe hybridization solutions specific for this test. Program parameters may be changed if desired. The operation is started. This completes the labor by a human being.

The automatic steps completed by the x-y-z traverse robotic arms of the apparatus of the present invention, as guided by the programming of the

central computer, generally begins with the pipetter transferring a portion of the biological sample to each well of the nucleic acid plate followed by the required reagents. The plate centrifuge and indexer positions the nucleic acid plate in the mixer/shaker position where the reagents and sample are mixed. The nucleic acid plate is then centrifuged as part of a wash process. The nucleic acid sample plate is then positioned at one of the 50 to 100 °C incubators where the plate is incubated for 1 hour. Additional reagents and wash steps are performed on the nucleic acid sample plate.

Next, the nucleic acid sample is modified. When the modification is amplification by PCR, a portion of each sample in the nucleic acid titer plate is then transferred to a well of the PCR titer plate along with the required reagents. The thermal cycling portion of the PCR process is then performed by positioning the PCR plate at each of the programmable incubators, set at 55 °C, 72 °C, 94 °C and 4 °C. This sequence is repeated many times. Other types of manipulations that can be performed by this sub-system include, but are not limited to, restriction enzyme cutting, ribozyme cutting and hybridization.

A portion of each sample in the PCR plate is then transferred to a column of one of eight gel cassettes, each containing 12 columns (totally 96). The gel cassettes are transferred into and out of the gel phoresis system where they are processed together.

After processing, each gel cassette is then transferred to the optical reading module of the gel phoresis system where the pattern is scanned and transferred to the control system for analysis using the gel analysis software module.

In the device of the present invention, samples are individually transferred from plate 1 to plate 2 for the addition of reagents, solutions, enzymes, nucleotide primers and probes required for identification. Transfer between modules is in coordination with the reagents and tests being run in

the chamber of the instrument. This feature allows the genetic specimen to undergo further treatments without transfer to another vessel.

Advantages to performing multiple steps or methods in one vessel include: (1) standardization of accurate assay results (2) less technician skill and less technician preparation and handling time required and thus lower test cost (3) more convenient sample collection and (4) less human error in switching samples or labels. Current methods require a technician to prepare a sample and transfer it to another container or a gel together with other specimens. A specimen may go through several container changes during processing, and each container change is a possible source of error in identifying a patient specimen or sample source. The same 96 well plate in this invention contains the patient specimen or sample throughout the entire processing until it is electrophoresed. But then, it is a robotic arm that transfers the sample to the proper position, thereby minimizing error.

The device of this invention facilitates quick results of nucleic acid-based diagnostics and genetic surveillance and detection. Although the discussion and examples herein are directed primarily to DNA analysis, it is clear that the device of the present invention may be used with RNA with equal facility.

Each kind of labeled probe that hybridizes to the target nucleic acid is detected according to the nature of its label molecule. The number of aggregates of detection signals corresponds to the number of original target sequences directly.

Different fluid treatments may be applied automatically in series to the gel electrophoresis module. The ability to automatically change the solution saturating the gel cassette is not currently available. The instrument provides processor-controlled fluid delivery to individual gel cassettes. An equivalent electrical current is supplied to each gel cassette by design of the circuits.[2]

Here, both related and unrelated specimens may be grouped together in the same non-standardized gels. In this invention, related specimens may be processed and compared with both a standard run with each gel.

Furthermore, the automated system represents versatility in applications. Different protocols can be programmed into the control center of the apparatus for each specific diagnostic or nucleic acid identification test. Gel size and composition will be adapted to perform a particular kind of assay. Spatial enumeration of signal identification positions of nucleic acids during repetitive probing may be determined in the gel cassettes.

As illustrated in the schematic of FIG. 3, the automated system has great flexibility in the inclusion and ordering of methods used. After sample preparation any one of several fundamental methods are performed first: electrophoresis or manipulation, including hybridization, enzyme restriction or amplification. Detection of the nucleic acid may occur after treatments for any one of the methods. A particular test can involve one or more of the methods before detection, in any order.

In summary, nucleic acid analysis depends upon one or more of the methods of manipulation, hybridization and electrophoresis, all of which may be performed using the modules according to an embodiment of the present invention. The automated system for nucleic acid-based diagnostics herein incorporates one or more of these methods in a given order depending upon the nature of the specimen and the quantity of nucleic acid in a particular type of specimen. Microprocessor controlled processing starts with a sample preparation phase. Lysing and deproteinizing treatments are performed automatically to prepare the sample specimen after it is loaded into the instrument. The application of treatments that follow are programmed to perform methods appropriate and prearranged for a particular diagnosis.

Accordingly, an object of the present invention is to provide a system for fast, low cost, high volume, non-contaminated and consistent automated nucleic acid analysis of multiple biological samples.

Another object of the present invention is to provide a modular apparatus wherein any of the sub-systems, i.e., sample preparation, electrophoresis, or manipulation, can be removed.

A further object of the present invention is to provide a system which can accommodate capacity loads within the range of one to 96 samples, or that can accommodate more than one probe per run.[3]

A further object of the present invention is to concentrate specimen nucleic acids or amplified products thereof, for detection of their presence or for further manipulation for a diagnosis.

A further object of the present invention is to provide a barrier to evaporation of solutions during processing.

A further object of the present invention is to provide a system which is adaptable to dispensing different quantities of different reagents for saturating specimens quickly with a series of solutions automatically.

A further object of the present invention is to provide a system wherein airflow and heating regulate and monitor temperature and humidity.

A further object of the present invention is to provide an apparatus and methods for automatically detecting nucleic acid sequences for the determination of the identity of microorganisms or pathogens in humans, plants and animals.

Another object of the present invention to provide an apparatus and methods for automatically detecting nucleic acid sequences for the determination of a genetic relationship, such as paternity or species identification, or for the determination of potential donors of organs or tissues.

Another object of the present invention is to provide an apparatus and method for automatically detecting nucleic acid sequences for use in forensic determinations or for protecting the blood supply.

Another object of the present invention to provide an apparatus and methods for automatically detecting nucleic acid sequences for the analysis of genetic diseases in humans, plants and animals.

These and other objects, features and advantages of the present invention will become apparent after a review of the following detailed description of the disclosed embodiments taken in connection with the accompanying drawing figures and the appended claims.

Brief Description of the Figures

FIG. 1 is a schematic drawing of the apparatus of the present invention for fast automatic processing and analysis of nucleic acids of biological specimens.

FIG. 2 is a schematic drawing of an electrical circuit closed by the controller.[4]

FIG. 3 is a schematic diagram showing some of the various analyses and methods for which the present invention may be used.[5]

Detailed Description

The present invention comprises an apparatus for mechanically preparing nucleic acids from a biological sample, manipulating the nucleic acids, electrophoresing the nucleic acid fragments, and analyzing the data. The apparatus minimizes human labor and thereby increases output and minimizes error and cost. The system comprises removable sub-systems for performing each task of specimen preparation, manipulation and electrophoresis.

As used herein, amplification is defined as a means to biochemically increase a nucleic acid mass. Target nucleic acid means those molecules containing a designated genetic sequence. Separation of nucleic acids by size utilizing electrophoresis is performed in a hydrogel supplied with an electrical current. Hybridization refers to the binding of complementary nucleic acids sequences, one partner of which carries a label whose signal can be detected. If amplification is used alone or follows hybridization it is understood that the primers or sequences used in binding targets or nucleotides for amplification may also carry a label.

The biological samples are processed in stepwise treatments that expose, modify, and detect the presence or absence of genetic entities in each sample. The nucleic acids or other desired biological components are treated by one or more techniques, such as, amplification, restriction enzyme cutters, electrophoresis, analyte-receptor binding or hybridization, as selected for the desired analysis. Different primer and polymerase molecules may be used to replicate the nucleic acids in the sample. The system also includes a heating and a passive cooling system that will provide rapid thermal cycling for denaturation and amplification protocols so requiring them, or maintain constant temperatures for isothermal amplification, restriction enzyme or hybridization protocols.

A preferred embodiment 10 having three sub-systems is shown in FIG. 1. The first sub-system robotically prepares nucleic acid from biological samples, such as, extracting genomic DNA from whole blood and removing undesired components, e.g. cell wall material, proteins, etc. The first subsystem is called a primary tube sampler system 12 and comprises a 96 sample tube carrousel module 14 for shaking and centrifugation, a 37 °C plate incubation heating block 16, and a sample identification barcode reader module 18. Additional biological samples that may be automatically processed in this sub-system include any biological sample or environmental sample suspected of containing biological material. Blood, plasma, serum,

saliva, cerebrospinal fluid, lymph, urine, homogenous tissue, cell cultures, viruses, water, and soil are examples of sample material, but the process is not limited to these materials. Even though the current standard size of a well plate includes 96 wells, different size well plates and gel cassettes can be used in this invention, depending on the requirements of a particular laboratory.

The nucleic acid present in the sample that has been introduced into an individual well is separated from the other cellular particles or sample debris by lysing solutions and thorough washing following standard protocols found in the art, such as, the well-known laboratory manual of Sambrook et al., Molecular Cloning: A Laboratory Manual, second edition, Cold Spring Harbor Laboratory Press, New York (1989) (which is incorporated by reference herein).

The present invention includes any possible coating of the well surfaces with selected biomolecules, natural or synthetically-manufactured, by chemically attaching them to the 96 well plate. For well plates made of glass, a known standard method of binding biomolecules to surfactants is with sulfonyl chlorides (Nilsson et al., In W. B. Jakoby (Ed.) Methods in Enzymology, Vol. 104, 1984, Academic Press, Inc., Orlando Fla.). For well plates made of polypropylene or polystyrene, chemical attachment may be by hydrophobic binding to their phenyl groups. The purpose of adhering molecules to the well plates is to facilitate the processing of genetic detection.

The second sub-system is connected to the first sub-system. After the nucleic acid is prepared in the first sub-system, The second sub-system manipulates and modifies the nucleic acid according to the pre-programmed methods appropriate and prearranged for a particular diagnosis. Types of manipulations include, but are not limited to, enzyme restriction, amplification, denaturation, hybridization and ribozyme cutting. Types of testing include, but are not limited to, infectious disease diagnosis, identity

testing, cancer diagnostics and predisposition testing. The second sub-system is called the titer plate centrifuge and indexer system 20. In a preferred embodiment the titer plate centrifuge and indexer system 20 comprises a titer plate centrifuge and two position rotor module 22, three programmable 50 to 100 °C incubation modules 24, 26, 28, one programmable 4 to 37 °C incubation module 30, and a mixer/shaker module 32. The range of temperatures for the incubators may be varied as required for a particular laboratory. Also included is a thermal cycler unit 34. The thermal cycler unit 34 can be used for PCR amplification, such as, random amplification of polymorphic DNA of extracted genomic DNA.

In alternative embodiments of each of the sub-systems, a heater may be required. For example, racks in the sample tube carrousel module hold the gel cassettes and well plates. The racks are also designed in such a way that they position the well plates for heating/cooling. An example of a heater (not shown) that might be used is a 2-dimensional resistant heater (Minco, Minneapolis, Minn.) laminated to an aluminum plate. Preferably, aluminum fins are added as an extension of the aluminum plate to expand the heaters' heat-sinking capacity. The heater outputs are controlled by a programmable microprocessor and sensor inputs. The racks position the well plates in the instrument modules so that each well plate is at an intimate distance from a heated surface. Heat from the heater source (not shown) is transferred to the wells or gel from the heater (not shown) whose surfaces closest to the racks may have protruding sections in a pattern that permits intimate contact between the well plate bottoms or gel and the heater. An alternative heating method may be from resistance-type heating coils incorporated into specified locations in the rack. In either case, the intimate distance means that surfaces of the heater and well plates or gel actually touch, or not, either continuously or intermittently, in a way that heat is convected through an air cushion layer from the protruding sections of the heat sink to the well plates

or conducted from them or the rack directly to specific areas of the well plate bottom.

The heating system causes the samples within the well plate and/or gel to attain a set point temperature (ramping) in a specified time period, maintain that temperature set point for a specified time period (soaking) and repeat throughout a programmed temperature profile. Temperature control in the well plate is necessary for providing conditions for the kinetics of specific biochemical reactions, i.e., specific enzyme activity or annealing or disassociation of complementary nucleic acids.

A possible type of manipulation by the second sub-system employs random primers for PCR amplification.

Other types of manipulation by the second sub-system can be predictive (1) in cancer when in vivo gene amplification means a more aggressive malignancy or (2) in viral infections to distinguish latent viruses from active infection. A given degree of amplification of target nucleic acid in the second sub-system will distinguish locations that represent a few copies of original target from many copies of target. The difference in amplitude of these signals, and construction of a total signal by summing individual signals, reflects a more accurate quantitative answer for each specimen as opposed to measuring a single amplitude for total signal of each specimen. In addition to improving measurement of signals over background noise, the method is useful to distinguish individual particles/cells having a few copies of a target nucleic acid from those with many copies.[6]

Another use for the second sub-system 20 of the present invention is detecting a particular sequence variation which may indicate individual identity, disease susceptibility or disease state. Nucleic acid sequences are excellent molecular probes because of the complementarity of primer and probe sequences to target nucleic acid for the purpose of amplification and

hybridization. Similarly the recognition sites of restriction endonucleases are nucleic acid-sequence specific. Restriction fragment length polymorphisms (RFLP's) are the result of restriction endonuclease cleavage and require electrophoretic size fractionation.

To perform non-random amplification and/or hybridization, a solution containing primer, nucleotide and polymerase molecules is added to the wells. The DNA is amplified by rounds of primer extension of target DNA. A short time is allowed for annealing of one or more primer pairs (a pair is defined as two primers that border opposite ends of a linear target DNA and are complementary to the opposite DNA strands) at an appropriate temperature. The number and choice of primer pairs and the number of replication cycles will vary according to the assay being performed. The sequence of a target nucleic acid must be known to determine a system to be used for detection. As more sequence information becomes available, the choice of primers for any one system may be changed to reflect a conserved genetic region and improve the specificity of detection. New technology may improve fidelity of primer annealing and DNA polymerization to allow accurate detection by incorporating labeled nucleotides in the amplification step, thus eliminating the need for a separate hybridization step in the detection process.

After the gene amplification reaches the level needed for detection by the hybridizing probe the probes are added to the wells. The hybridizing probe consists of single-stranded DNA complementary to, but shorter than, the DNA target sequence and has one or more label molecules attached. The choice of nucleotide sequences for the hybridization probe reflects the same considerations stated for primer sequences.[7]

An alternate procedure for the second sub-system 20 involves primer pairs back to back along a target sequence in order to extend longer targets

efficiently. The number of primer pairs in a linear or nested series may vary to accommodate the size-length of DNA required to immobilize the amplified segments during treatment. This alternative requires a ligase to incorporate each primer covalently to the linear molecule at its 5-prime end and the ligase needs to be thermo-resistant. In a particular system, such an enzyme would need to be isolated from nature, if it has not been already isolated.[8]

Another alternate procedure for the second sub-system 20 involves adding the hybridization probes during an amplification phase. When single-stranded, labeled probe molecules are incorporated into the growing chains, they become part of the amplified DNA and sequential hybridization is not necessary. Since the process time is dramatically reduced in simultaneously amplifying and labeling the DNA, this step is desired. An enzyme for joining single strand nicks as described in the preceding paragraph is also necessary in order to insure the target sequence was labeled unambiguously over a background of randomly-primed, amplified DNA.[9]

The third sub-system 36 is connected to both the first 12 and second 20 sub-systems. The third sub-system 36 is for separating fractions of the nucleic acid using gel electrophoresis. In some nucleic acid analyses, the nucleic acid may be transferred directly from the first sub-system 12 to the third sub-system 36 in order to make an initial determination about the nucleic acid. In other instances, the nucleic acid will be manipulated by the second sub-system 20 before the nucleic acid is run on the gel. In either case, the nucleic acid is mechanically transferred from the previous sub-system to the third sub-system 36 by the robotic arm of the pipetter system. The third sub-system 36 is called the gel electrophoresis separation system 36. In a preferred embodiment the gel electrophoresis separation system 36 comprises an eight cassette transport and processing module 38 and an

optical reading module 40 (or gel scanning unit). The gel electrophoresis separation system 36 uses pre-cast gel cassettes for repeatable separations. In alternate embodiments, more or fewer gel cassettes are employed, depending on the requirements of the particular laboratory.

In standard electrophoresis the prepared sample is manually loaded in the gel for electrophoresis, and the gel, or the nucleic acids in it, are manually handled for hybridization and detection. A feature of the gel electrophoresis separation system 36 of the present invention is that, after loading a gel into the third sub-system 36, the physical and chemical handling of the gel is automated within the instrument. Prepackaged, prepared gels for electrophoresis are loaded into the third sub-system 36 at the beginning of the operation. Here, the gel is kept "fresh" inside the cassette transport and processing module 38 until it is needed, then transferred to the optical reading module 40 for data analysis.

The purpose of the scanner 40 is to convert image data from the gel to a digital form for computer interface. Scanning identification signals are incorporated into the final stage of analysis. Each gel is exposed to the scanner 40, which reads the signals. The location and number of original target molecules present in the sample are reported by the signal. The scanning apparatus 40 is interfaced with the microprocessor to give quantitative (location of signal) and qualitative (strength of signal) measurements. A representation of signal measurements made *in situ* may be printed out. Scanner input data entered into a microprocessor matches the sample number with the identity of the sample and is further processed to determine the profile of the total sampled population. Software for the processor enables raw data of the sample to be structured with other information on the sample source and compared to a database.

In the second sub-system 20 either specific restriction endonucleases, ribozymes (non-protein RNA molecules that cut and resplice RNA into genetic messages) or polymerases may be introduced into the wells to act

upon the nucleic acids. The endonucleases break linear DNA into restriction fragment polymorphisms. Polymerase molecules, together with DNA or RNA primers, are used to expand a selected DNA or RNA fragment population. When run through the gel electrophoresis separation system 36, the fragments move toward the anode according to their size. Subsequent staining or hybridization within the gel enables the identification of specific band patterns. Amplification products may be identified by electrophoretic separation and non-specific nucleic acid staining; but in some cases hybridization probes are necessary to distinguish them from spurious amplification products which cause ambiguities.

The purpose of the electrical current in electrophoresis within the device of the present invention is to fractionate and concentrate the macromolecules by size. Electrophoretic mobility of specific DNA restriction fragments, RNA messages or amplified nucleic segments are then compared with those similarly treated from another specimen. For example, specimens from two or more individuals may be compared for paternity identification. Forensic specimens may be compared to specimens from suspects. Family groupings may be compared for markers of genetic disease. Tumor specimens may be compared to standards for classification.

An additional purpose for the use of two or more gel cassettes is to separate and compare functions or samples between two or more patient specimens. Alternatively one gel section may serve one function and the second gel section may serve another function. Examples of different functions are: (1) cleansing nucleic acids in a specimen from interfering biological material; (2) amplifying the nucleic acid fragments; (3) hybridizing a labeled probe to the nucleic acids; (4) fractionating nucleic acids according to size by electrophoresis; (5) comparing an internal standard on the matrix with an unknown; and (6) comparing band patterns to indicate related individuals.

An air flow system is built into the thermal chamber of the cassette transport and processing module 38 to cool the gel cassettes during electrophoresis and prevent uneven heat build-up. The closed position of the gels during electrophoresis prevents evaporative loss of buffer.[10]

Each of the sub-systems requires a means to mechanically pipette the biological samples, reagents used in the three sub-systems, waste generated in the three sub-systems, and the nucleic acids. Therefore, the present invention also comprises a pipetter system 42. The pipetter system 42 is a robotic 96 well plate mover comprising an X-Y-Z traverse 44, 46, 48 and probe module 50,[11] a pipetter pump module 52, and a pipetter waste module 54. The x-y-z traverse 44, 46, 48 is a mechanical (for example, robotic) arm that may be used to apply precise volumes of particular reagents sequentially to each well's receiving area. Either the mechanical arm 44, 46, 48 may move between the sub-systems 12, 20, 36 or the sub-systems 12, 20, 36 may rotate past the mechanical arm 44, 46, 48. The system also includes pipetter pumps 52 to provide suction and release in the pipette for administering and removing substances from the well plates.

Additionally, the present invention also comprises a reagent and pipette storage system 56. The reagent and pipette storage system 56 comprises a reagent and sample refrigeration storage module 58 kept at 4 °C, and a pipette storage module 60. In FIG. 1, the reagent and sample refrigeration storage module 58 holds 20 vessels and the pipette storage module 60 holds 2000 pipette tips.[12] Examples of reagents include, but are not limited to, lysing and denaturing solutions; neutralizing solutions; reagents for amplification; reagents for hybridization; and hybridization wash solutions. A particular vial's contents can be changed for each diagnostic test. Custom-made primers (for amplification) and labeled probes

(for hybridization detection) can be made for any known target nucleic acid nucleotide sequence. Different wells may be treated for different target sequences in the same operation, therefore different reagents, such as, primer probes would be added to each well.

An optional part of the apparatus of the present is a programmable central microprocessor, called a control system invention (not shown), for controlling the apparatus and analyzing the data in the processed gels. The control system comprises a digital computer module, a laboratory interface module, a hardware interface module, a system control software module, a data management software module, and a gel analysis software module. Steps known in the art may be used by the control system to detect the particular component once run on a gel. Protocol requirements, such as, timing and volume of sequential treatment fluids delivered to each of the well specimens; regulation of and rapidly changing the temperature of each of the well specimens; drying cycles; water rinses; and solution treatment sequences may be programmed into the control system as necessary.

In summary, the molecular processing is accomplished by processor-controlled commands for the sub-systems 12, 20, 36 directing the molecular manipulations and providing the necessary microenvironment in the individual sub-systems 12, 20, 36. The command system for controlling the microenvironment is chosen for specific gene probe sequences or different types of specimens and consists primarily of duration, pH and temperature of treatments containing standard and custom-made solutions. A user of the system needs only to enter the desired program of treatments into the processor, load appropriate reagents to each sub-system 12, 20, 36, load the samples into well plates and the well plates into the primary tube sampler system 12. The processor then automatically selects predetermined appropriate reaction conditions (time, duration, treatment solution, solvent or reagent) for the sample type, and initiates the appropriate commands, in the

appropriate sequence, and at the appropriate times to obtain protocol conditions which allow the molecular manipulations.

The system's flexible programming allows use of the device for research and clinical applications in which only one or two of the sub-systems are required. Some examples of the different uses are preparation of large-sized DNA or intact chromosomes from cultured cells or organisms for other DNA manipulations, amplification of target DNA for other DNA manipulations, and probe hybridization in which nucleic acids have been size-fractionated by electrophoresis. If the RNA in the sample is the target to be amplified, the sample is treated with reverse transcriptase to make a nucleic acid complement of the RNA just prior to the amplification step.

The ability to monitor specific nucleic acid sequences in biological material allows surveillance of genetic changes and fate-monitoring of known genetic changes. Both the lack of sensitivity of current probes and the labor-intensive preparation of the biological material has slowed application of recombinant DNA technology. The sensitivity of gene probes is increasing but some biological samples, especially those from the environment or a large population base, require massive sampling and screening to monitor the dispersal of the target gene. This method, which eliminates tedious sample preparation by automating the procedure, expands the ability to study gene competition, stability, dispersion and evaluate efficacy of new, recombinant DNA product treatments.

The device and method of the present invention may be used to determine the presence and/or amount of a selected target component. Such processing is useful for example, in determination of the presence of viruses or biological components such as sequence-specific nucleic acids in fluids, or tissues of plants and animals, in microorganisms, or in environmental samples; the identity ("finger-printing") of an individual from the sample; the presence of genetic similarities, diseases, or abnormalities; or mapping of genes.

There are several advantages of the present invention. For example, the three sub-systems 12, 20, 36 are modular and each sub-system 12, 20, 36 can be removed or added to these or other sub-systems. The flexible assay format can be tailored to an individual laboratory's needs. In a preferred embodiment, the apparatus 10 has a fully automatic operation that completes 96 sample analyses in under eight hours. The data is free from contamination problems common with manual methods. The apparatus employs a non-toxic reagent system. The barcode reader 18 allows continuous sample identification from primary tube to result. The apparatus of a preferred embodiment having three sub-systems 12, 20, 36 is a compact, integrated eight foot by four foot floor standing system.

The operation of the complete system 10 begins with an operator placing one to 96 barcode labeled whole biological samples, such as blood, into carousel. Two empty 96 well titer plates are placed in the rotor of the plate centrifuge and indexer 22. One plate is used for the nucleic acid extraction while the other is used for the manipulation of the nucleic acid, such as PCR amplification. New gel cassettes are placed in the gel phoresis and reader system 36. Necessary reagent vials and a generous supply of pipette tips, for example, 2,000 tips, are placed in the reagent refrigeration storage module 58 and the pipette storage module 60, respectively, of the reagent and pipette storage system 56. The desired diagnostic test is chosen on the computer. The computer may provide instructions for checking or inserting reagents, such as enzyme solutions or probe hybridization solutions specific for this test. Program parameters may be changed if desired. The operation is started. This completes the labor by a human being.

The automatic steps completed by the x-y-z traverse 44, 46, 48 of the apparatus 10 of the present invention, as guided by the programming of the central computer, generally begins with the pipetter transferring a portion of the biological sample to each well of the nucleic acid plate followed by the required reagents. The plate centrifuge and indexer 20 positions the nucleic

acid plate in the mixer/shaker 32 position where the reagents and sample are mixed. The nucleic acid plate is then centrifuged as part of a wash process. The nucleic acid plate is then positioned at one of the 50 to 100 °C incubators 24, 26, 28 where the plate is incubated for 1 hour. Additional reagents and wash steps are performed on the nucleic acid plate.

Next, the nucleic acid is modified. When the modification is amplification by PCR, a portion of each sample in the nucleic acid titer plate is then rotated to a well of the PCR titer plate along with the required reagents. The thermal cycling portion of the PCR process is then performed by positioning the PCR plate at each of the programmable incubators 24, 26, 28, 30, set at 55 °C, 72 °C, 94 °C and 4 °C. This sequence is repeated many times.

A portion of each sample in the PCR plate is then transferred to a column of one of eight gel cassettes, each containing 12 columns (totally 96). The gel cassettes are transferred into and out of the gel phoresis system 36 where they are processed together.

After processing, each gel cassette is then transferred to the optical reading module 40 of the gel phoresis system 36 where is the pattern is scanned and transferred to the control system for analysis using the gel analysis software module.

The nucleic acid analyzer of the present invention is automated. Each of the steps of sample preparation, manipulation, electrophoresis and data analysis is completed by the apparatus 10. Additionally, the methods and apparatus 10 of the present invention have a maximum throughput of 96 samples every seven hours, or 864 samples every three days. This fast data output is further made desirable because of low associated costs. Labor in processing the samples and analyzing the data is ten minutes per day. Also desirable is the cost of the equipment to perform the analysis, which can be as low as one tenth the cost of currently available equipment. All of these costs translate into low costs to the consumer per nucleic acid analysis.

Further adding to the desirable aspects of fast sample processing and low costs are significantly decreased problems with sample contamination, and low variations in sample processing and data analysis that are all performed by the same machine that may only occasionally need to be calibrated. Additionally, there are fewer errors from misidentified vials.

Turning to FIG. 2, the electrical system for nucleic acid analysis broadly comprises[13] The design of racks to hold the gel cassettes in which electrophoresis is used will position the gel in such a way that the gel completes an electrical circuit. The electrodes on each rack of a plurality of racks holding the gel, are positioned in such a way that applied current can pass through each gel saturated with an electrical conducting buffer. Positive and negative connections are located on opposite ends of each gel and connected via contact leads to positive and negative terminal blocks on the rack.

The rack has electrical connections fitting into corresponding connections in the instrument when the racks are in position in the instrument. The rack is thus connected to a power supply in the automated instrument. The present invention so equipped will provide an equivalent electrical current through all gel cassettes. In such racks electrical connections from the anode bus bar and cathode bus bar lead to each individual gel cassette, an anode to one end of the gel and a cathode to its opposite end. Electrical wire connections are appropriately sheathed with insulating material where no current conductance is desired. Interlocks and lid locks will be placed at all points where an operator may inadvertently come into contact with the electric field.

FIG. 3 is a schematic diagram showing some of the various analyses and methods for which the present invention may be used.

With the present invention, nucleic acids can be analyzed by less sophisticated image analysis systems than the more expensive and time consuming analyses currently being used. Thus, the present invention is valuable as a cost-effective prescreening of specimens for genetic sequences, and for diagnosing an "active versus latent" disease stage, and a "pathological versus non-pathological" disease state.

It is to be understood that automated processing begins with sample preparation and ends with the analysis of the test results. It is further to be understood that standard reagents and reaction conditions may be used for the various sample treatment steps, such as amplification, electrophoresis and hybridization. In the following examples, polymerase chain reaction (PCR) is shown as the preferred method of amplification using thermal cycling, but amplification is not limited to PCR or to thermal cycling because isothermal methods are known. Previous research described in U.S. Patent No. 5,451,500, herein incorporated by reference (especially examples 2 and 5-16), has demonstrated amplification in agarose gels by PCR with Taq polymerase. The addition of more primer molecules during PCR as they are used retards formation of undesirable primer dimers. Although not discussed in detail herein, standard techniques including immunostaining for analysis of polypeptides or other cellular components in gels may be performed with the device of the present invention.

This invention is illustrated by the included examples, which are not to be construed in any way as imposing limitations upon the scope thereof. On the contrary, it is to be clearly understood that resort may be had to various other embodiments, modifications, and equivalents thereof which, after reading the description herein, may suggest themselves to those skilled in the art without departing from the spirit of the present invention and/or the

scope of any later included claims. Protocols and recipes are those found in standard laboratory manuals, such as, Sambrook et al.[14]

Example 1

Genomic DNA Extraction from Whole Blood for ??[15]

Nontoxic Organic Solvent Protocol performed in Sub-System 1:

(A) Isolation of Nuclei with Surfactant

To each well of the 96 well plate, 0.5 ml of whole blood sample is mixed with 0.5 ml of lysis solution by the plate shaker. The supernatant is centrifuged and discarded. 1 ml of lysis solution is added. The mix is shaken [Shaker mix[16]], followed by centrifugation and the supernatant is discarded. Another 1 ml of lysis solution is added. The mix is again shaken, centrifuged and the supernatant discarded.

(B) Enzyme Digestion: To Detach Protein from DNA

200 µl of enzyme solution and 10 µl of protease solution are added to each well. The well plate is incubated 37 degrees C for 1 hour. The well plate is cooled back to room temperature.

(C) DNA Precipitation

300µl of sodium iodide solution and 0.5 ml of isopropanol are added to each well. The mix is shaken [Shaker mix[17]], followed by centrifugation and the supernatant discarded. 1 ml of washing solution A is added. The mix is shaken [Shaker mix], followed by centrifugation [and the supernatant discarded?][18]. 1 ml of washing solution B is added and followed by shaker mix and centrifugation. [and the supernatant discarded?][19]. The pure precipitated genomic DNA is saved for the next step.

Random Amplification of Polymorphic DNA Protocol: RAPD: performed in Sub-System 2:

(A) Reagent Preparation for use in Protocol

Combine RAPD analysis beads with dNTP, BSA & Taq Enzyme. Prepare primer I: 5′ primer and Primer II: 3′ primer; Magnesium Chloride Solution: 10 mM; and pure patient DNA template samples from extraction step reconstituted in 1 ml of purified water.

(B) PCR Protocol

The 96 well plate is filled with one RAPD bead per well. 15 µl of purified water, 2 µl of Primer 1, 2 µl of Primer II, 4 µl of magnesium chloride solution and 2 µl of patient DNA template sample are added to the corresponding well. Also included is one control well without DNA template. Shaker mix.[20] 20 µl of mineral oil is added to the top of the solution or the plate is sealed. The 96 well plate is transferred to the thermal cycler. The following thermal cycle program is set as: (a) thermal equilibration: 94°C , 30 min., (b) 35 thermal cycles : 94°C , 1 min. --- 55°C , 1 min. --- 72°C , 1 min., and (c) thermal equilibration: 72°C , 10 min. Storage for next step is set at 4°C .

Standard Agarose Gel Protocol: performed in Sub-System 3:

(A) Reagent Preparation

Necessary reagents include self-contained, bufferless, precast, high-resolution DNA agarose gel cassette with electrophoresis system; DNA ladder markers; and PCR product from corresponding patient DNA template. These are loaded into the apparatus.

(B) Gel Electrophoresis Protocol

The precast gel cassette is inserted into the electrophoresis unit. Then 20 µl of each of the followings are loaded into the corresponding well

position of the gel: (a) DNA ladder markers; (b) PCR product from the control well; and (c) PCR product from the corresponding patient DNA template. The timer is set and the gel is processed. The gel cassette is scanned and the result of the gel separation is analyzed by the control system.

Example 2

Genomic DNA Extraction from Whole Blood for Cancer Detection[21]:

Example 3

Genomic DNA Extraction from Whole Blood for Identity Testing[22]:

Example 4

Genomic DNA Extraction from Whole Blood for Predisposition Testing[23]:

The disclosures of all patents and publications cited in this application are hereby incorporated by reference in their entireties in order to more fully describe the state of the art to which this invention pertains.

Although the present process has been described with reference to specific details of certain embodiments thereof, it is not intended that such details should be regarded as limitations upon the scope of the present

invention except as and to the extent that they are included in the accompanying claims.

Claims

What is claimed is:

1. An apparatus, comprising:

a first sub-system for robotically preparing nucleic acid from biological samples;

a second sub-system removably connected to the first sub-system for modifying the nucleic acid, wherein the nucleic acid is mechanically transferred from the first sub-system to the second sub-system;

a third sub-system removably connected to the first and second sub-systems for gel electrophoresis of the nucleic acid, whether the nucleic acid is modified or not, wherein the nucleic acid is mechanically transferred from the previous sub-system to the third sub-system;

means to mechanically pipette the biological samples, reagents used in the three sub-systems, waste generated in the three sub-systems, and the nucleic acids electrically connected to each subsystem; and

a programmable central microprocessor for controlling the apparatus and analyzing data obtained by the apparatus electrically connected to each subsystem and to the means to mechanically pipette the biological samples.

wherein the three sub-systems are modular and each sub-system can be removed or added to these or other sub-systems.

2. The apparatus of claim 1 wherein the modification of the nucleic acids by the second sub-system is amplification.

3. A method for studying genetic material of biological samples employing an automatic apparatus, comprising the steps:

a) placing the biological samples into a first sub-system of the automatic apparatus;

b) choosing a type of analysis to be performed on the nucleic acid by the automatic apparatus;

c) placing necessary reagents and supplies for the analysis in the first sub-system and in a second sub-system removably connected to the first sub-system and in a third sub-system removably connected to the first and second sub-systems;

d) starting the automatic apparatus wherein

i) the first sub-system robotically prepares nucleic acid from the biological samples,

ii) the automatic apparatus optionally transfers the nucleic acid from the first sub-system to the second sub-system, wherein the second sub-system robotically modifies the nucleic acid, and

iii) the automatic apparatus mechanically transfers the nucleic acid from the previous sub-system to a third sub-system, wherein the third sub-system robotically gel electrophoreses the nucleic acid and analyzes data obtained by the gel electrophoresis, wherein several types of analyses are pre-programmed into a central processor of the automatic apparatus electrically connected to each of the three sub-systems; and

e) reviewing the analysis obtained by the gel electrophoresis.

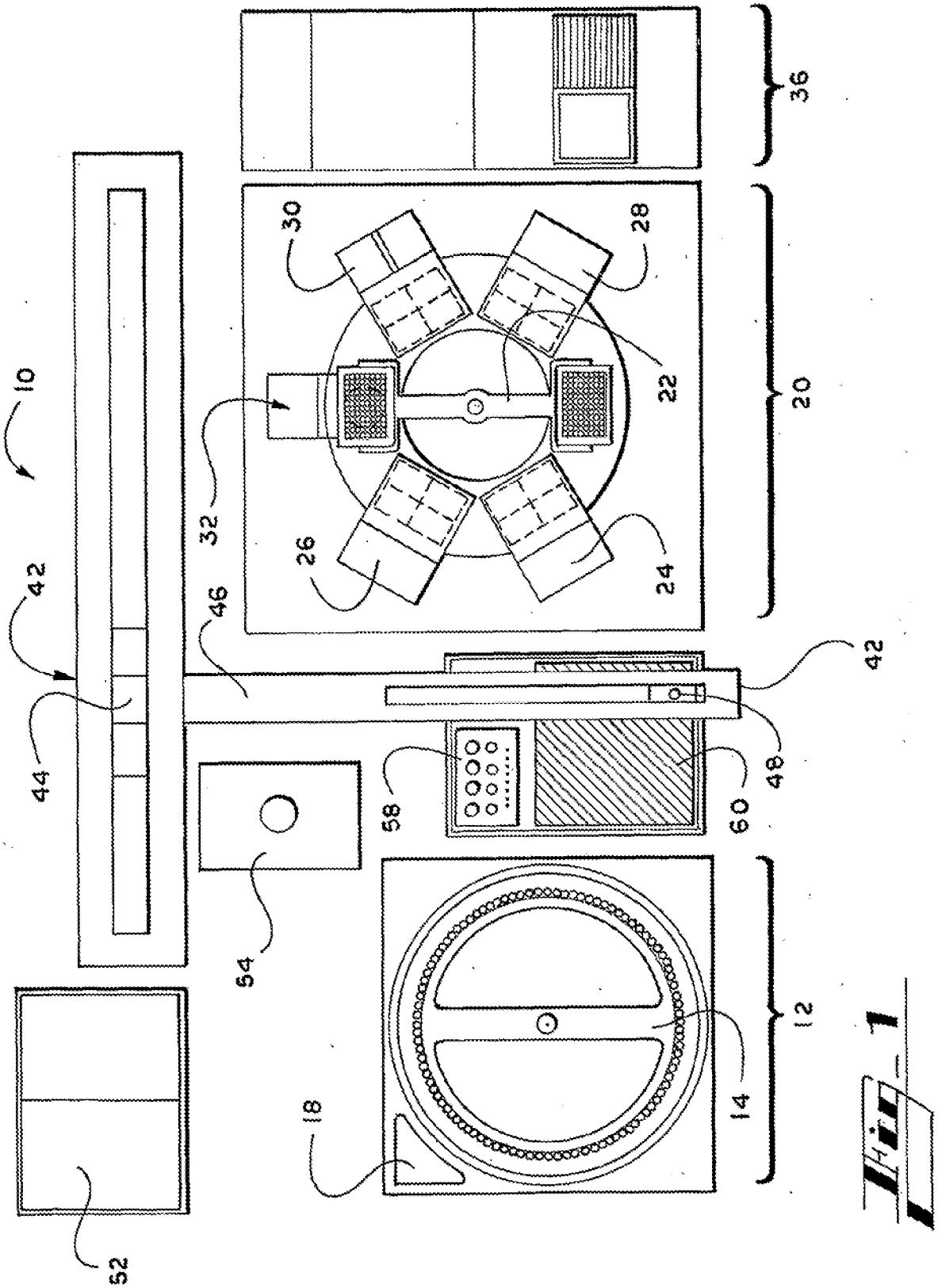

Fig. 1

Fig 2

- Sub Systems
  - Pipetter, xyz motion, Cleaning Station
  - Primary Tube Sampler
  - Reagent and pipette tip storage
  - Centrifuge - (2 position rotor)
    - → Mixing Station
    - → Heating and cooling Stations
  - Gel Electrophoresis System
    - → Reader

Extraction
- 1st Sub System
- Pipetter, xyz motion, Cleaning Station
- Primary Tube Sampler
- Reagent and Pipetter Tip Storage
- Centrifuge
  - → Mixing Station

PCR
- 2nd Sub System
- Heat / Cooling Stations for Centrifuge

Electrophoric Analysis
- 3rd Sub System
- Gel Electrophoresis System

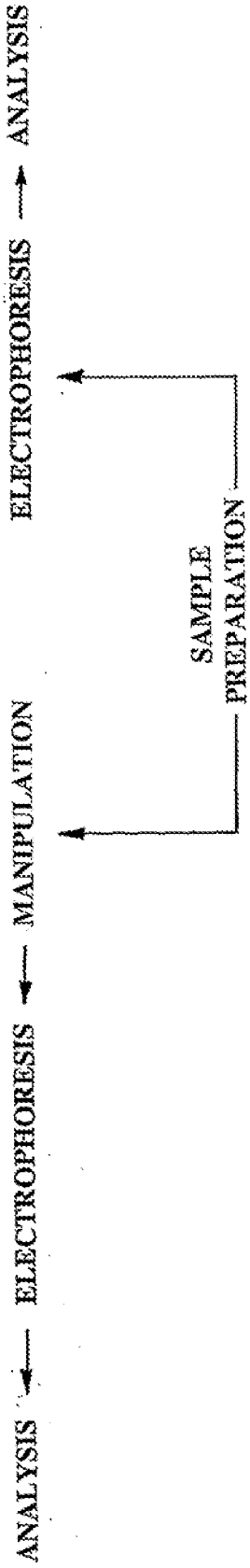
ANALYSIS
ELECTROPHORESIS
MANIPULATION
SAMPLE PREPARATION
ELECTROPHORESIS
ANALYSIS

Fig. 3

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/09588

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N35/02 G01N1/28 G01N27/447 //C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N B01L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, EPO-Internal, INSPEC, COMPENDEX

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BELGRADER PHILLIP ET AL: "Automated sample processing using robotics for genetic typing of short tandem repeat polymorphisms by capillary electrophoresis" LABORATORY ROBOTICS AND AUTOMATION,US,VCH PUBLISHERS, NEW YORK, vol. 9, no. 1, 1 February 1997 (1997-02-01), pages 3-7, XP002090124 ISSN: 0895-7533 page 4 -page 6; figure 1	1-3
Y	DE 38 05 808 A (EUROP LAB MOLEKULARBIOLOG) 7 September 1989 (1989-09-07) column 1, line 23 -column 2, line 7 column 5, line 8 - line 40; figures	1-3

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 August 2000	22/08/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hodson, M

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/09588

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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Form PCT/ISA/210 (continuation of second sheet) (July 1992)

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International Application No
PCT/US 00/09588

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